of antifolates in general. YETI provides an easy method to analyze the energy of ligand–protein interactions and permits quick optimization of these interactions.

In summary, our modeling studies show that Tyr-31 can exist in either orientation with respect to PTX binding. However, when Tyr-31 is perpendicular to Phe-34, its phenol oxygen contributes to a hydrophilic environment for PTX, which suggests that the perpendicular orientation may be favorable. In the perpendicular position, the phenol oxygen of Tyr-31 hydrogen bonds with water-424; however, in mammalian DHFR this potential hydrogen bond is lost because residue 31 is a phenylalanine. As such, advantages of the perpendicular position for Tyr-31 are lost for the mammalian enzymes. These studies indicate that there are two slightly different perpendicular (e.g., $\tau_1/\tau_2 \simeq -131/-105°$ and $-98/-105°$) conformers of PTX which have two modes of binding that are independent of the orientation of Tyr-31. While these binding studies show a preference for a perpendicular PTX conformation (i.e., $\tau_1/\tau_2 \simeq 90/90°$), which differs from that observed in the crystal structure, there is less than 2.5 kcal/mol energy difference between them.

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Supplementary Material Available: Tables of anisotropic thermal parameters, hydrogen-bonding geometry, and atomic partial charges (2 pages); lists of structure factor calculations (14 pages). Ordering information is given on any current masthead page.

Kinetic Solvation Pressure: A Measure of Environmental Effects on Reaction Rates. 1. Application to Hydrophobic Systems[1,2]

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Abstract: A quantity termed kinetic solvation pressure is defined as $(\partial \Delta G^{\ddagger}/\partial \bar{V})_T$, where $\bar{V}$ is the reactant molar volume. It is identified with the difference in the amount of isothermal work, per unit volume expansion necessary to create a solvation cavity in a particular medium, upon transition-state complex formation. The quantity was evaluated for the hydrolysis of carboxylic esters mediated by different hydrophobic catalysts and was found to be equal to $+26$ J cm^{-3} for the acid-catalyzed hydrolysis of *n*-alkyl acetates in water solvent; but it becomes negative when macro- or supramolecular acids were used as catalysts: -16 J cm^{-3} for Dowex 50W-X2, -43 J cm^{-3} for poly(styrenesulfonic acid), -64 J cm^{-3} for dodecylsulfuric acid micelles. These results suggest the action of hydrophobic forces in enhancing the catalytic power of the supermolecules, relative to aqueous hydrogen ion. No such effect is seen in aqueous acetone or when more hydrophilic acetates are used as substrates. Kinetic solvation pressure for enzyme-catalyzed ester hydrolysis is five times more negative than for the resin system, indicating the full action of hydrophobic forces in the catalytic process.

The effect of solvent on reaction rates is a fundamental and yet unsolved problem in chemical kinetics, because formal calculation of partition functions for reactants and transition-state (TS) complexes in the liquid state is too difficult.

The traditional approach has been to correlate activation free energies (or log *k* values) with physical properties of the solvent such as dielectric constant, viscosity, and Hildebrand solubility parameters[3] or with a vast array of empirical solvent parameters.[4] The electric field of solute molecules causes changes in the solvent liquid structure in their immediate microenvironment and, therefore, the direct use of bulk solvent properties seems not likely to be adequate.

I propose in this paper an alternative way to obtain a view of solvent–solute interactions, with further applications to macro- and supramolecular catalysts.

Statement of the Model: Solvation Pressures. Let a chemical process occur in a certain solvent at constant temperature and pressure and, further, let the molecules be dissected into a reactive group and an appendage (e.g., an alkyl chain). Figure 1 gives

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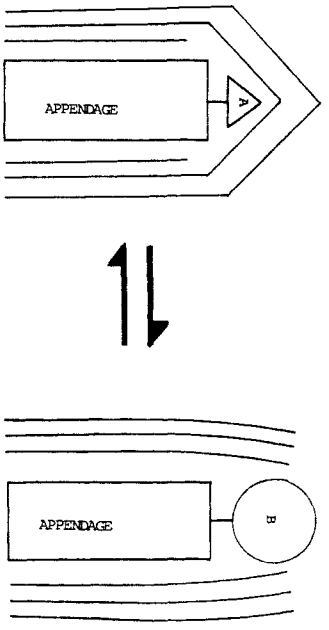

Figure 1. Schematic representation of solvation change in chemical transformations.

a diagrammatic picture of the solvent reorganization that occurs in dilute solution when the solute molecules change from the thermodynamic state A to state B. Note that the main idea is that the molecular structure of the appendage remains constant between states A and B but the solvent structural organization around the solute molecules after the change has taken place does not.

We want to vary the appendage from case to case and measure the effect of this variation on $\Delta G(A,B)$, in the hope that the changes in $\Delta G(A,B)$ will "report" on the changes in microenvironment of the appendage between states A and B. What feature of the appendage should be varied? Significant structure theory suggests that the partial molal volume of the appendage is the desired property.

Solids expand on melting, but nearest neighbors remain at essentially the same distance.[5] The significant structure model of the liquid state[6] assumes that a collection of material particles and "holes" make up a liquid system. A hole persists if the local kinetic energy density, which makes a region expand, is balanced by the opposing potential energy density (internal pressure) tending to bring about the collapse of the hole. Interestingly, Chandler[7] has applied the formalism of quantum mechanics to solvation phenomena, assuming the liquid state to be formed from a collection of material particles and "cavity particles". Whenever a solute dissolves, a place has to be created in the solvent to accommodate that solute. The energy required to make the niche depends on the cohesion of the solvent and the volume of the hole.[8] The substrate partial molal volume ($\bar{V}_R$) seems indeed to be the microscopic variable needed.

According to this view, $(\partial \bar{G}_R/\partial \bar{V}_R)_{T,P}$ must measure the isothermal work per unit volume expansion necessary to disrupt the solvent liquid structure, in order to adjust the size of cavities in which the solute molecules are located. It can be envisioned as the "pressure" exerted by the solvent liquid structure on the solute molecules filling the cavities. Thus, $(\partial \bar{G}_R/\partial \bar{V}_R)_{T,P}$ gives the medium effect on the stability of solute molecules and must be a function of the medium cohesiveness (internal pressure) and therefore has pressure units (energy/volume). I propose to call $(\partial \bar{G}_R/\partial \bar{V}_R)_{T,P}$ the *solvation pressure*. Since most chemical processes in solution are carried out at constant pressure, the subscript P will be dropped out only for convenience, but it must always be kept in mind that solvation pressures are defined at constant pressure. My interpretation of the physical meaning of $(\partial \bar{G}_R/\partial \bar{V}_R)_T$ is favored by the finding that the difference between free energies of solution of normal alkanes, alkenes, and 1,ω alkadienes in aqueous and in hydrocarbon media is found to be a linear function of the number of carbon atoms in the hydrocarbon chain.[9a]

$(\partial \bar{G}_R/\partial \bar{V}_R)_T$ can be obtained experimentally by measuring the chemical potential of a series of homologous solutes in which the appendage has essentially the same structural features but different molecular sizes, as for example a family of *n*-alkyl derivatives, in which $d\bar{V}_R$ arises from the steady increase in alkyl chain length.

Molecular Origin of the Solvation Pressure. Leffler and Grunwald indicate that the total free energy of a system can be approximately computed fron intrinsic, entropy, and pairwise interaction contributions (eq 1):[10]

$$G/RT = \sum n_i B_i + \sum n_i \ln C_i + (1/V)\sum\sum n_i n_j \beta_{ij} + \text{higher order interactions} \quad (1)$$

In the first term, n_i is the number of moles of the ith component and the B_i are constants that depend only on their chemical nature (RTB_i is the sum of the free energy of formation of the reactive group and of the appendage plus the free energy of interaction of the two molecular moieties). The reader can easily identify the second term in eq 1 as the contribution from the entropy of mixing. The third term contains β_{ij} coefficients, which account for pairwise interactions among the constituents in the system of volume V.

For a two-component system, the solute partial molal free energy (solute chemical potential) can be calculated as[10]

$$\bar{G}_R' = (\partial G/\partial n_R)_{T,n_s} = RT[B_R + \ln C_R + 1 + (2\beta_{RR} - \bar{V}_R)C_R + (2\beta_{RS} - \bar{V}_R)C_s - \bar{V}_R(\beta_{ss}C_s^2 + \beta_{RR}C_R^2 + 2\beta_{RS}C_RC_s)] \quad (2)$$

In order to specify a standard state for solute R, one can take the solvation process

$$R(\text{gas}) \rightarrow R(\text{solvent S})$$

Then, the molar free energy of solute R with respect to its ideal gas state at a given temperature T and standard pressure, $\bar{G}_R$, equals

$$\bar{G}_R = \bar{G}_R' - \bar{G}_R(\text{gas})$$

$$\bar{G}_R = \text{cratic contribution} + \text{interactions term}$$

This specification of $\bar{G}_R$ eliminates the intrinsic term, and the entropy of mixing becomes a constant at given T, P, and C_R. The interactions term is given by $[1 + (2\beta_{RR} - \bar{V}_R)C_R + (2\beta_{RS} - \bar{V}_R)C_s - \bar{V}_R(\beta_{ss}C_s^2 + \beta_{RR}C_R^2 + 2\beta_{RS}C_RC_s)]$.

The corresponding value for solvation pressure of medium S upon solute R is obtained by differentiation with respect to $\bar{V}_R$ (eq 3).

$$(\partial \bar{G}_R/\partial \bar{V}_R)_T = -RT[(C_s + C_R) + C_s^2\beta_{ss} + C_R^2\beta_{RR} + 2C_sC_R\beta_{RS}] \quad (3)$$

Equation 3 indicates that the solvation pressure of medium S is defined by the extent of solute–solute and solute–solvent interactions as well as by the strength of solvent–solvent interactions.

For the case of a dilute solution, the solvation pressure becomes

$$(\partial \bar{G}_R/\partial \bar{V}_R)_T = -RTC_S(1 + C_R/C_S + C_S\beta_{SS} + 2C_R\beta_{RS}) \quad (4)$$

where the C_R second-order term in eq 3 is neglected because of the high dilution condition.

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Equation 4 clearly says that $(\partial \bar{G}_R/\partial \bar{V}_R)_T$ gives mainly the "solvent cavity effect"[4] on the solubility of solute R, in a system in which high dilution prevents solute–solute interactions from playing a significant role in the stability of solute R in solution.

Equilibrium Changes in Solvation Pressure. It follows from the previous section that the solvent effect on the position of the equilibrium (system in state A) $\rightleftharpoons$ (system in state B) is given by the corresponding differences in reactant and product solvation pressures:

$$\text{equilibrium change in solvation pressure} = (\partial \Delta G^\circ/\partial \bar{V})_T \quad (5)$$

where ΔG° is the standard free energy change of the process and $d\bar{V}$ notes the steady increase in volume of the appendange in the reactants and products. The appendage-reactive group model states that the volume of molecules i in a homologous series is given by the sum of appendage volume ($\bar{V}_{app}(i)$), the volume of the reactive group ($\bar{V}_{reac}$), and the volume of the rest of the molecules, that is

$$\bar{V}_A(i) = \bar{V}^A_{app}(i) + \bar{V}^A_{reac} + \bar{V}_{rest}$$

$$\bar{V}_B(i) = \bar{V}^B_{app}(i) + \bar{V}^B_{reac} - \bar{V}_{rest}$$

$$\bar{V}_B(i) = \bar{V}_A(i) + (\bar{V}^B_{reac} - \bar{V}^A_{reac})$$

$$\bar{V}_B(i) = \bar{V}_A(i) + \text{constant}$$

$$d\bar{V}_B(i) = d\bar{V}_A(i) = d\bar{V}$$

An explanation on how to obtain $\partial G/\partial \bar{V}$ values is given in the Experimental Section (vide infra).

Comparison with Traditional Formulation of Solvent Effects. There is a point that deserves further comment. Unlike other approaches, in which the solvent effect on equilibria is given relative to a standard medium,[10] $(\partial \Delta G^\circ/\partial \bar{V})_T$ gives the effect of a particular environment on the relative stability of the molecules in the final state B, relative to the situation in the initial state A.

In other approaches, a comparison is typically made of ΔG(A,B) values (ΔG°) in a given solvent 2 relative to the observed quantity in a reference medium 1. Such a comparison gives the free energy of transfer of the molecules in state B from solvent 1 to solvent 2, minus the analogous transfer free energy of molecules in state A:

$$\Delta G_2(A,B) - \Delta G_1(A,B) = G_2(B) - G_2(A) - G_1(B) + G_1(A)$$

$$\Delta G_2(A,B) - \Delta G_1(A,B) = [G_2(B) - G_1(B)] - [G_2(A) - G_1(A)]$$

In contrast to KSP values, this kind of information emphasizes the transfer process between two media. The equilibrium change in solvation pressure gives the effect of the solvent on the thermodynamics of the final state B, relative to the effect of the same solvent on the thermodynamics of the initial state A.

Kinetic Solvation Pressures. Transition-state theory assumes that the specific rates of chemical reactions are proportional to the concentration of TS complexes, which is determined by the free energy difference between TS and reactant state. In analogy to the equilibrium change in solvation pressure, a kinetic change in solvation pressure (or, more briefly *kinetic solvation pressure*) is defined as $(\partial \Delta G^\ddagger/\partial \bar{V})_T$, where $\bar{V}$ is the substrate molar volume.[45] The physical meaning of the kinetic solvation pressure can be understood as follows:

$$(\partial \Delta G^\ddagger/\partial \bar{V})_T = (\partial G^\ddagger/\partial \bar{V})_T - (\partial G^\circ/\partial \bar{V})_T \quad (6)$$

where $G^\ddagger$ and G° are the partial molar free energies of TS complexes and reactants, respectively. As was shown in a previous section, $(\partial G^\circ/\partial \bar{V})_T$ gives the extent of solvent–reactant interaction. In a theoretical sense, $(\partial G^\ddagger/\partial V^\ddagger)_T$ measures the solvent–TS interaction. However, the quatity that appears in eq 6 is not $(\partial G^\ddagger/\partial V^\ddagger)_T$ but instead $(\partial G^\ddagger/\partial \bar{V})_T$. This turns out not to cause difficulty for the following reason. Through chain rule derivation

$$(\partial G^\ddagger/\partial \bar{V})_T = (\partial G^\ddagger/\partial V^\ddagger)_T(\partial V^\ddagger/\partial \bar{V})_T \quad (7)$$

In general, one has that $(\partial V^\ddagger/\partial \bar{V})_T$ has a value close to 1 because $d\bar{V}$ arises from the steady increase in the appendage molecular size, along the homologous series. Therefore, $(\partial G^\ddagger/\partial \bar{V})_T = (\partial G^\ddagger/\partial V^\ddagger)_T$, which is the transition-state counterpart of $(\partial G^\circ/\partial \bar{V})_T$. This leads to eq 8.

$$(\partial \Delta G^\ddagger/\partial \bar{V})_T = (\partial G^\ddagger/\partial V^\ddagger)_T - (\partial G^\circ/\partial \bar{V})_T \quad (8)$$

Since $(\partial G/\partial \bar{V})_T$ measures the effect of the medium on the stability of a given chemical species in solution, one obtains from eq 8 that $(\partial \Delta G^\ddagger/\partial \bar{V})_T$ measures the difference of such effect between the TS and the reactant state; that is, the derivative accounts for the environmental effect upon TS complex formation. The term kinetic solvation pressure (KSP) is therefore an appropriate term to denote $(\partial \Delta G^\ddagger/\partial \bar{V})_T$.

Negative KSP values indicate smaller cohesiveness of the medium in the neighborhood of the TS complex than in the neighborhood of the reactant molecules. Positive KSP values indicate the opposite situation.

Application of the Model to Kinetics in Regular Solutions. KSP is connected with a major concept of solution chemistry: internal pressure. Scatchard and particularly Hildebrand[6] developed equations for the activity coefficients of nonelectrolytes as solutes, within the framework of regular solutions. If the solution is dilute, one has that

$$RT \ln \gamma_R = \bar{V}_R(\delta_R - \delta_S)^2 \quad (9)$$

where γ_R, $\bar{V}_R$, and δ_R are the activity coefficient, molar volume, and the well-known Hildebrand solubility parameter of solute R, and δ_S is the analogous quantity for the solvent. δ^2 is the cohesive energy density (proportional to internal pressure). The term $\Delta_R = (\delta_R - \delta_S)^2$ measures the feasibility for R and solvent to mix to produce a regular solution.

These ideas have been applied to the kinetic problem of solvent influence.[11] Transition-state theory indicates that the medium effect on the rate of chemical processes is introduced via the activity coefficients of the reactant molecules R's and of the TS complex (eq 10). k_{ideal} is the reaction rate constant in ideal solution.

$$k/k_{ideal} = \prod \gamma_R/\gamma_\ddagger \quad (10)$$

This equation can be transformed to eq 11.

$$\ln k = \ln k_{ideal} + \sum_R \ln \gamma_R - \ln \gamma_\ddagger \quad (11)$$

Introducing the Scatchard-Hildenbrand relation yields eq 12.

$$RT \ln k = RT \ln k_{ideal} + \sum_R \Delta_R \bar{V}_R - \Delta_\ddagger V^\ddagger \quad (12)$$

Finally, for a family of homologous solutes, one obtains by simple differentiation eq 13.

$$(\partial \Delta G^\ddagger/\partial \bar{V})_T = -RT(\partial \ln k/\partial \bar{V})_T = (\Delta_\ddagger - \Delta_R) \quad (13)$$

These algebraic manipulations indicate that KSP is indeed a direct measurement of the medium effect upon TS complex formation. Equation 13 predicts a value of zero for an ideal-gas reacting system, because $\Delta = 0$ due to the lack of molecular interactions.

The chemical processes of interest in this paper and in biochemistry in general are those taking place in aqueous media and other organized systems. Medium cohesiveness in water and in micelle solutions, protein solutions, vesicles, or microemulsions is the resultant of many complex interactions. Because of this complexity, measures of medium cohesiveness such as KSP cannot always be reliably dissected into all their contributing terms for such media. Nevertheless, it remains true that the size of a solute defines the extent of interaction necessary to form and maintain the supramolecular organized particles against the thermal motion of the molecules.[12] Therefore, whether its reliable dissection can be accomplished or not, KSP should be a useful guide to microenvironmental effects even in the complex media.

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Table I. Second-Order Rate Constants for the Homogeneously (H_2SO_4) and Heterogeneously (Resin) Catalyzed Hydrolysis of a Group of *n*-Alkyl Acetates at 37 °C in 62% Aqueous Acetone and in Water Solvent[a]

AcOR	H_2SO_4–aq acetone, $10^5k_H/s^{-1}$ M^{-1}	resin–aq acetone, $10^5k_r/s^{-1}$ mol^{-1} L	resin–water, $10^5k_r/s^{-1}$ mol^{-1} L
Me	9.1 ± 0.3	25.27 ± 0.02	51 ± 1
Et	7.8 ± 0.4	16.7 ± 0.8	54 ± 1
n-Pr	6.7 ± 0.3	11.2 ± 0.3	60.0 ± 0.1
n-Bu	5.7 ± 0.2	7.6 ± 0.4	71.3 ± 0.5
n-Am	4.6 ± 0.2	4.53 ± 0.06	

[a] Error limits are standard deviations from the mean of three or five runs.

Table II. Second-Order Rate Constants for the Homogeneously (H_2SO_4) and Heterogeneously (Resin) Catalyzed Hydrolysis of a Group of Esters in Water Solvent at 35 °C

ester	$10^4k_H/s^{-1}$ M^{-1}	$10^4k_r/s^{-1}$ mol^{-1} L
ethyl formate	52.2 ± 0.5	102 ± 1
ethyl acetate	3.16 ± 0.04	4.78 ± 0.08
ethyl isobutyrate	0.730 ± 0.003	2.43 ± 0.09
ethyl thioglycolate	2.10 ± 0.03	4.61 ± 0.01
ethyl phenylacetate	0.36 ± 0.05	2.30 ± 0.04
γ-butyrolactone	3.325 ± 0.005	8.1 ± 0.2
2-ethoxyethyl acetate	1.15 ± 0.04	2.92 ± 0.02
acetonyl acetate	0.63 ± 0.01	1.29 ± 0.01
1-glyceryl actate	1.45 ± 0.3	1.88 ± 0.01

This work presents results on the KSP values derived from data on the hydrolysis of esters bearing hydrophobic appendages, catalyzed by macro- and supramolecular catalysts, partly taken from the literature and partly the result of new experiments. The KSP values will be discussed in terms of the effect of the immediate surrounding medium on the stability of TS complexes.

Experimental Section

Materials. All esters were common chemicals taken from the shelf and distilled prior to use. Acetone (Baker) was distilled over CaO/$KMnO_4$.

The ion-exchange resin Dowex 50W-X2 was from Fluka in the Na^+ form and was converted to the H^+ form as described previously.[14] The resin is a strongly acidic (SO_3H) polystyrene gel with 2% divinylbenzene as cross-linking agent and 4.28 ± 0.02 mmol of SO_3H residues/g.

Kinetic Measurements and Data Treatment. The kinetic runs were carried out by titrating the acid formed as described in ref 14. The data gave good first-order plots, whose slopes were determined by the least-squares method. Error limits are standard deviations from the average from three or five experiments.

$(\partial\Delta G^{\ddagger}/\partial\bar{V})_T$ was calculated as $-2.303\,RT\,[\partial \log (k)/\partial\bar{V})_T$. The values of $\bar{V}$ were calculated from the esters' densities given in the CRC Handbook of Chemistry and Physics. $(\partial\Delta G^{\ddagger}/\partial\bar{V})_T$ values for other data taken in the literature were calculated by using 17 cm^3/mol as the molar volume of the CH_2 group.[15]

Results

Alkyl Acetates. The hydrolysis of the esters obeyed the kinetic law given in eq 14, where C_r is the ratio of the amount of moles

$$d[RCO_2H]/dt = k_rC_r[ester] \qquad (14)$$

of resin acidic groups used to the volume of the liquid phase. Table I gives the second-order rate constant at 37.0 °C for the homogeneously (H_2SO_4) and heterogeneously (resin) catalyzed hydrolysis of *n*-alkyl acetates in 62% aqueous acetone and in water solvent. The resin-catalyzed process shows faster specific rates than the H_2SO_4 reactions in the mixed solvent.

Figure 2 shows that log k_r values decrease linearly with the molar volume of the substrates in the mixed solvent, and the same is observed in the homogeneous experiment. The resin reaction in water solvent shows the opposite behavior.

For the one-phase reaction, $(\partial\Delta G^{\ddagger}/\partial\bar{V})_T = 25.7 \pm 0.9$ J cm^{-3}, and for the biphasic reactions, the quantity equals 62 ± 2 J cm^{-3} in aqueous acetone but −15.3 ± 0.5 J cm^{-3} in water solvent.

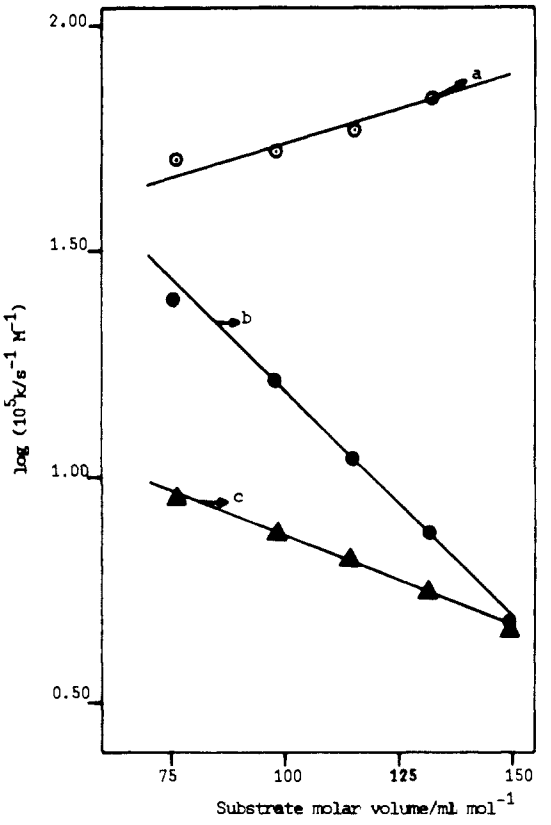

Figure 2. Effect of substrate size on the acidic hydrolysis of *n*-alkyl acetates. (a) water, Dowex catalyst; (b) 62% aqueous actone, Dowex catalyst; (c) 62% aqueous actone, H_2SO_4.

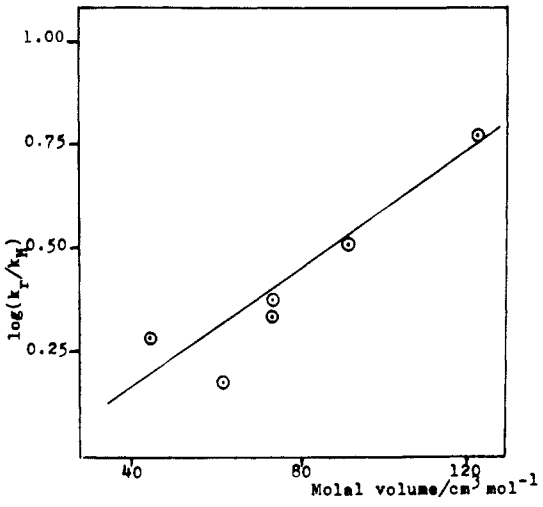

Figure 3. Effect of substrate size on the Dowex-catalyzed hydrolysis of a group of ethyl esters.

Ethyl Esters. The second-order rate constants for the resin-catalyzed hydrolysis of a group of ethyl esters at 35.0 °C are given in Table II. The substrates were chosen in such a way that the acyl groups were the side chains in glycine, alanine, valine, proline, phenylalanine, and cysteine.

Figure 3 shows a linear relationship between log (k_r/k_H) and the molar volume of the corresponding amino acids.[16] The $(\partial\Delta G^{\ddagger}/\partial\bar{V})_T$ value for the resin-catalyzed hydrolysis of the RCO_2Et has to be obtained through k_r/k_H values, because the introduction of different structures in the acyl section causes

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Table III. $(\partial \Delta G^\circ/\partial \bar{V})_T$ Values for the Transfer of Alkyl Moieties from Water to Hydrophobic Phases

process (ref)	$(\partial \Delta G^\circ/\partial \bar{V})_T$/J cm^{-3}
$H_2O \rightarrow$ hydrocarbon (9a, 21, 22, 23)	−200 ± 24
$H_2O \rightarrow$ β-lactoglobulin (9a)	−261 ± 5
$H_2O \rightarrow$ bovine serum albumin (9a)	−212 ± 5
$H_2O \rightarrow$ CTAB micelles (22, 24)	−120 ± 14
$H_2O \rightarrow$ SDS micelles (22, 25)	−116 ± 18
$H_2O \rightarrow$ Dowex 50/K$^+$ form (26)	−15 ± 6

[a] This value is the average for the transfer of hydrocarbons, alkanols, alkanoic acids, and alkyl ethers.

changes in reactivity due to intrinsic properties of the R groups (steric and electronic) and to acyl medium interactions (solvation). Equation 15 leads to the calculation of $(\partial \Delta G^\ddagger/\partial \bar{V})_T$ for the resin-catalyzed reaction relative to the situation in water solvent.

$$-2.303RT[\partial \log (k_r/k_H)/\partial \bar{V}]_T = [(\partial \Delta G^\ddagger/\partial \bar{V})_T]_r - [(\partial \Delta G^\ddagger/\partial \bar{V})]_H \quad (15)$$

Discussion

Solvation Pressures in Hydrophobic Systems. The hydrophobic effect is the most important factor in the organization of the supermolecular arrays of living matter.[9a] Since the medium effects to be discussed in this paper are dependent on hydrophobic forces, a preliminary account on solvation pressures (SP) in systems where these interactions are important will first be presented. Table III gives a few ΔSP values, calculated from data found in the literature, which deal with the distribution of nonpolar solutes between water and hydrophobic solvents.

Interesting features are noted when the value for the transfer of alkyl chains is taken as a point of reference. This value of ~−200 J cm^{-3} represents the characteristic ΔSP associated with transfer of a nonpolar solute from pure water to a typical hydrophobic environment. This ΔSP can then be compared with changes for transfer of solutes from water to environments that are less simple and well-characterized.

The change in solvation pressure for binding of alkyl chains to bovine serum albumin (BSA, ΔSP = −212 J cm^{-3}) and to β-lactoglobulin (β-LG, ΔSP = −261 J cm^{-3}) is somewhat more negative than for hydrocarbon solvents. This is because, while the solvation cavity in the hydrocarbon liquid phase has to be created, this cavity already exists in the protein molecules. It is known that the hydrophobic binding site of β-LG can accommodate a hydrocarbon with $\bar{V}$ of the order of up to 200–230 cm^3/mol, whereas BSA has several hydrophobic patches of smaller sizes.[9a] This means a higher degree of molecular restriction for the solute in the binding cleft of BSA. This restriction should give less positive $(\partial \Delta S^\circ/\partial \bar{V})_T$, and thus a less negative ΔSP than for β-LG.

The restricted translational and rotational freedom of individual molecules in micelles should give rise to a less exergonic ΔSP for the incorporation of hydrophobic solutes from aqueous media. This is due to the entropic factor that arises from the more mechanically restricted structures. This is shown to be the case with CTAB and SDS micelles, where solute–solvent interactions in both cases have ΔSPs that are only half as much as the value observed for liquid hydrocarbon phases. The rigid ion-exchanger matrix is at the end of the spectrum with ΔSP = −15 J cm^{-3}. The result indicates that it takes advantage inefficiently of the forces involved in the transfer of hydrophobic solutes.

Hydrolysis of Esters. The acid-catalyzed hydrolysis of alkyl acetates is insensitive to the polar effects from the alkyl moieties,[17,18] its kinetics being governed by steric effects alone.

Table IV. Calculated $(\partial \Delta G^\ddagger/\partial \bar{V})_T$ Values for the Hydrolysis of Esters Accelerated by Macromolecular and Supramolecular Catalysts

reaction	KSP/J cm^{-3}	ref
Acidic Catalysis		
AcOR + H_2O (water, H_2SO_4)	25 ± 4	this work
AcOR + H_2O (62% aq acetone, H_2SO_4)	25.7 ± 0.9	this work
AcOR + H_2O (62% aq acetone, HCl)	25 ± 3	38
Saponification in Water Solvent		
AcOR + NaOH (water)	24 ± 2	38
HCO_2R + NaOH (water)	24 ± 2	38
RCO_2R' + NaOH (water)	26 ± 7	18
Saponification in Mixed Organic–Aqueous Solvents		
$PhCO_2R$ + NaOH (56% aq acetone)	51 ± 5	38
AcOR + NaOH (62% aq acetone)	82 ± 9	38
$AcOCH_2R$ + NaOH (70% aq acetone)	74 ± 5	17b
AcOR + NaOH (17% aq dioxane)	54 ± 6	38
$AcOCH_2CH_2R$ + NaOH (70% aq dioxane)	63 ± 3	39
AcSR + NaOH (43% aq acetone)	63 ± 7	38
AcSR + NaOH (62% aq acetone)	61 ± 6	38
Neutral Hydrolysis		
CF_3CO_2R + H_2O (water)	157 ± 10	44
CF_3CO_2R + H_2O (50% aq dioxane)	148 ± 11	17b
CF_3CO_2R + H_2O (70% aq acetone)	127 ± 13	17b
$HOCH_2CH(OH)CO_2R$ + H_2O (water)	8 ± 2	43
AcOR + H_2O (62% aq acetone, Dowex 50W-X2)	62 ± 2	this work
AcOR + H_2O (70% aq acetone, Amberlite IR 120)	108 ± 11	40
AcOR + H_2O (water, Dowex 50W-X2)	−15.3 ± 0.5	this work
AcOR + H_2O (water, Amberlite IR 120)	−12 ± 1	42
AcOR + H_2O (water, poly(styrenesulfonic acid))[a]	−43 ± 1	20
AcOR + H_2O (water, poly(styrenesulfonic acid))[b]	−55 ± 1	20
AcOR + H_2O (water, dodecylsulfuric acid)	−64 ± 1	19
Enzyme Reactions		
RCO-chymotrypsin + H_2O	−105 ± 18	33
RCO-chymotrypsin + H_2O	−126 ± 7	34
(AcNH)CHRCO-chymotrypsin + H_2O	−130 ± 10	35
RCO-trypsin + H_2O	−124 ± 15	34
triacylglycerol + H_2O (castor bean lipase from V_{max})	−136 ± 5	36
RCO_2Et + H_2O (horse liver esterase)	−200 ± 62	37
RCO_2Et + H_2O (pancreatic lipase)	−226 ± 43	37

[a] 100% sulfonation. [b] 60% sulfonation.

Table IV gives KSP values for a collection of rate data on ester hydrolysis obtained from the literature. The data show no ap-

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Table V. Calculated $(\partial \Delta G^{\ddagger}/\partial \bar{V})_T$ Values for Ester Hydrolysis Catalyzed at 25 °C by Sulfonic Acid Ion Exchangers of Different Degree of Cross-Linkage in 70% Aqueous Acetone[a]

% divinylbenzene	KSP/J cm^{-3}	% divinylbenzene	KSP/J cm^{-3}
1	63	8	99
4	81	20	112

[a] Calculated from data in ref 41.

preciable difference in the KSP between water solvent and aqueous organic media. This indicates that the microenvironmental effects on the stability of the positively charged TS complex are similar in water and in mixed organic systems. One possible interpretation, although others are possible, is that the transition-state microenvironment is essentially completely aqueous even in solvents containing a substantial organic component.

The situation for ester saponification in water solvent is the same as for the acid-catalyzed counterpart (as easily predicted), but the presence of an organic cosolvent alters significantly the KSP, raising its value by ~140%. This result indicates that solvation shells in the mixed solvents containing acetone or dioxane are harder to expand in the formation of the negatively charged TS complex than are shells made only of water molecules. The implication is also that, while the positively charged acidic hydrolysis TS favors a highly aqueous microenvironment, the negatively charged basic hydrolysis TS permits the organic component into its microenvironment.

A further interesting result from Table IV is that obtained from the data on the neutral hydrolysis of *n*-alkyl glycerates[43] and *n*-alkyl trifluoroacetates.[17b,44] For the glycerate esters, the TS complexes and initial glycerate molecules are both very waterlike. This means that the corresponding solvation pressures must be very similar in water solvent. A KSP value close to zero is therefore predicted, which is actually the case: 8 ± 3 J cm^{-3}.

In contrast, KSP is expected to be higher for the harder-to-solvate trifluoroacetates in relation to regular alkyl estesrs, which have KSP = 26 J cm^{-3}. The experimental result turns out to be 144 ± 15 J cm^{-3}.

Supermolecule Catalysis In Ester Hydrolysis. The fitting of substrate molecules in the microenvironment of active sites of macro- and supramolecular catalysts if of importance in chemistry and biochemistry. The catalytically successful ensemble depends on the kinds of interactions developed in the TS and, thus, could be related to the substrate molecular size, as well as to other molecular properties.

The study of the effect of substrate size in the hydrolysis of esters catalyzed by ion-exchange resins is an interesting case in which the chemical process occurs within the hydrophobic polymer matrix. These systems are useful models for biological events such as enzymic reactions and hormone–receptor binding, because the relative lack of molecular flexibility of the tridimensional array models a rigid biopolymer. The substrate molecules enter the resin through a diffusional process and then move through the polymer matrix until they reach the catalytic sites where reaction takes place. This last event is generally the rate-controlling step.[13]

The higher degree of constraint of TS complexes inside a resin phase, relative to homogeneous liquids, should be evident from the variation of reaction rates with substrate size, and KSP is precisely the mathematical quantity that measures this feature. If this quantity is positive, it indicates a tendency of the microenvironment to exclude TS complexes as their sizes increase, whereas the opposite results when the derivative is negative.

Table VI. Obstructive Effect on the Hydrolysis of Ethyl Acetate at 37 °C by the Degree of Cross-Linkage in Dowex Resins[a]

% divinylbenzene	$10^4 k_r$/s^{-1} mol^{-1} L
2	5.4 ± 0.1
8	4.97 ± 0.02
12	4.02 ± 0.07

[a] 10^4 k value for H_2SO_4 catalysis is 3.3 ± 0.1 s^{-1} M^{-1}.

Our data in Table IV and the results in Table V show that the reactivity also decreases with an enlargement of the substrate size for the resin-catalyzed hydrolysis of esters in mixed solvents. These solvents should be similar in polarity and hydrophobic character to the polymer matrix itself. Thus, the effect of relative rigidity can be roughly isolated. The reactions occur at greater ergonic expense relative to homogeneous processes (62 vs 25 J cm^{-3}), probably indicating the hindrance to solvent reorganization imposed by the rigid polymer matrix. This can be understood in terms of the high pressures developed inside resin matrices upon swelling,[27] which imply tighter molecular packing than in homogeneous liquid media due to the excluded volume of the polymer backbone.

Taniguchi et al.[19,20] studied the hydrolysis of *n*-alkyl acetates catalyzed by soluble poly(styrenesulfonic acid) and by dodecyl hydrogen sulfate micelles in water solvent and found the reaction rate to *increase* as the alkyl chain length grew. They argued that this effect arose from increased hydrophobic binding of the ester molecules to the catalyst structure. The negative value of KSP = −16 J cm^{-3} for the resin-catalyzed hydrolysis of carboxylic esters in water solvent suggests also the action of hydrophobic forces in substrate binding to the resin catalyst.[19,20,24]

Taniguchi and Mata-Segreda[28] showed that the apparent volume of activation for the resin-catalyzed hydrolysis of *n*-butyl acetate in water solvent was that expected for the transfer of the substrate from aqueous medium to the resin phase, plus the normal $\Delta V^{\ddagger}$ value observed in homogeneous systems.

In the mixed-solvent example discussed above, the water-structure breaking effect of acetone makes impossible the action of hydrophobic forces, and the nonspecific van der Waals forces in the system are not strong enough to concentrate the substrate molecules efficiently in the resin phase. These hydrophobic effects can also be depressed when more hydrophilic substrates react. The data in Table II show that the rate constants of resin-catalyzed hydrolysis of acetonyl acetate ($\bar{V}$ = 108 cm^3/mol), 1-glyceryl acetate ($\bar{V}$ = 111 cm^3/mol), and 2-ethoxyethyl acetate ($\bar{V}$ = 136 cm^3/mole do not correlate with the trend observed for the more hydrophobic *n*-alkyl acetates listed in Table I (e.g., propyl acetate, $\bar{V}$ = 114 cm^3/mol).

Table II shows the second-order rate constants for aqueous H_2SO_4 and Dowex-catalyzed hydrolyses of a group of ethyl esters, where the acyl moieties are the side chains of the hydrophobic amino acids glycine, alanine, valine, phenylalanine, proline, and cysteine. From the slope of the plot log (k_r/k_H) vs the molar volume of the corresponding amino acid, one obtains

$$-42 \pm 4 \text{ J cm}^{-3} = [(\partial \Delta G^{\ddagger}/\partial \bar{V})_T]_r - [(\partial \Delta G^{\ddagger}/\partial \bar{V})_T]_H$$

The above figure gives the ΔSP value for the transfer of the TS complex from water solvent to the resin microenvironment.

The results in Table IV also indicate that flexible linear soluble polymers and micelles are better catalysts than the rigid resin for the hydrolysis of esters. Their overall KSP values relative to the aqueous resin system are 28 and 49 J cm^{-3} more favorable. This effect must come from more negative entropies of substrate transfer for the reactions taking place within the resin phase, because actually lower $\Delta H^{\ddagger}$ values have been found for resin-catalyzed reactions than for their homogeneous counterparts.[29,30]

An additional experiment was done to test this hypothesis, in which the hydrolysis of ethyl acetate was studied with resins of different degree of cross-linkage. Table VI shows the expected rigidity effect. Nevertheless, the rate show from 22% to 64% greater value for the ion-exchanger reactions than for aqueous

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H_2SO_4 at the same temperature. Essentially, the same result was reported on the resin-catalyzed hydrolysis of *n*-butyl acetate.[28]

In summary, the accumulation of substrate molecules inside the polymer phase contributes to the resin additional catalytic power over aqueous hydrogen ions. The values given in Tables III and VI show that the rigid resin matrix takes advantage unefficiently from the forces involved in the transfer of hydrophobic substrates. The data support the correlation suggested above between catalytic power and catalyst molecular flexibility.

Kinetic Solvation Pressure in Enzyme Reactions. The catalytic power of enzymes can be accounted for in terms of two fundamental contributions: (1) a negative change in the solvation pressure of substrate molecules when transferred from the bulk of the medium to the active site, plus (2) the stabilization of the TS complex by the catalyst's microenvironment.

The first term can be obtained as $+RT(\partial \ln K_s/\partial \bar{V})_T$, where K_s is the enzyme–substrate complex dissociation constant. For many cases, $K_m = K_s$ and this contribution is therefore approximately equal to $+RT(\partial \ln K_m/\partial \bar{V})_T$.

The second contribution is $-RT(\partial \ln k_{cat}/\partial \bar{V})_T$. The model systems discussed above failed to give any important contribution to the second effect; most of its catalytic power over aqueous H^+ came from a modestly favorable partition of substrate molecules from the bulk aqueous medium to the catalytic pseudophase.

Contrary to the crude model systems, chymotrypsin can successfully stabilize the TS complex for the hydrolysis of a family fo $AcNHCHRCO_2Me$ esters.[35] The substrate binding contribution to the KSP is -182 J cm^{-3}, and the further TS complex stabilization by increased hydrophobic interaction leads to a contribution to KSP of -130 ± 10 J cm^{-3}.

Another interesting case is that of lipolytic enzymes that perform their catalytic actions at oil–water interfaces,[32] such as pancreatic lipase and horse liver esterase. These enzymes catalyze the hydrolysis of carboxylic esters when the latter are located at the hydrophobic interfaces of microemulsions. Thus, the initial state of the substrate is that of two-dimensional freedom at the interface.

The overall KSP for the hydrolysis of small esters by pancreatic lipase or horse liver esterase can thus be dissected into three contributions: (1) a value ΔSP(1) for the transfer of reactant-state nonpolar moieties (RS-X) from hydrophobic interfaces to bulk water medium, (2) a value ΔSP(2) for the transfer of RS-X's from bulk water to the hydrophobic catalytic sites of the enzymes, and (3) the actual amount for stabilization of the corresponding TS complexes, ΔSP(3).

(RS-X, interface) → (RS-X, bulk H_2O)

(RS-X, bulk H_2O) → (RS-X, enzyme)

(RS-X, enzyme) → (TS-X, enzyme)

(RS-X, interface) → (TS-X, enzyme)

ΔSP(1) can be considered equal to the opposite of the change in solvation pressure for the transfer of nonpolar molecules from water to micelles, +120 J cm^{-3}; ΔSP(2) can be reasonably equated to the −200 J cm^{-3} reference figure for water/hydrocarbon extraction. This would require ΔSP(3) = −130 J cm^{-3} to make an overall KSP of −210 J cm^{-3}, a figure similar to the KSP observed for triacylglycerol hydrolysis catalyzed by castor bean acid lipase under saturation conditions.[36] (−136 J cm^{-3}). The hydrophobic contribution of the catalytic power of resins, micelles, and polyions makes them interesting partial models for enzymic reactions, because the supramolecular arrays can also produce rate accelerations by bringing the reacting molecules together, by use of weak binding forces, in much the same way an enzyme uses a part of the intrinsic binding energy of the substrate (in the form of its TS complex) to pay for the entropy cost of assembling the reactive complex.[31] The rigid nature of the resin makes this repayment process less effective than in more flexible catalysts, as evidence by the magnitude of the KSP values.

A further characteristic of enzymes is that water molecules present within active sites in the reactant state can be displaced from the active site upon TS complex formation. Thus, it is appropriate to say that the catalytic efficiency should increase with the molecular flexibility of catalytic sites.

These results suggest that the natural evolutionary pathway in enzyme design is to produce flexible active-site structures, which take full advantage of the noncovalent binding forces developed in the TS.

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Thiamin Diphosphate Catalysis. Mechanistic Divergence as a Probe of Substrate Activation of Pyruvate Decarboxylase

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Abstract: Pyruvate decarboxylase is a thiamin diphosphate dependent enzyme that catalyzes the conversion of pyruvate to acetaldehyde and carbon dioxide. The substrate activates the enzyme, with kinetic patterns indicating a cooperative effect between two binding sites (Hill coefficient, 1.5). An alternative substrate, 3-fluoropyruvate, is converted to acetate, fluoride, and carbon dioxide. This reaction is not subject to activation, displaying normal Michaelis–Menten kinetics, but 3-fluoropyruvate activates the enzymic reaction of pyruvate. The dual reaction pattern was used as a probe of the cooperative phenomenon. Inhibition patterns show that 3-fluoropyruvate interacts with the enzyme at the same site as does pyruvate and that the affinities are similar. Since the reaction of 3-fluoropyruvate proceeds through a mechanism paralleling that of pyruvate up to the step in which carbon dioxide is lost, the step in the mechanism that is regulated occurs after the point at which the mechanisms diverge. The reaction of 3-fluoropyruvate produces enzyme-bound 2-(1-acetyl)thiamin diphosphate, which is readily hydrolyzed to produce acetate and holoenzyme, while the reaction of pyruvate produces the much less reactive enzyme-bound species 2-(1-hydroxyethyl)thiamin, which undergoes elimination of acetaldehyde to produce holoenzyme. These results suggest that the conversion of the complex of enzyme and 2-(1-hydroxyethyl)thiamin diphosphate to acetaldehyde and the holoenzyme is subject to allosteric control.

The thiamin diphosphate enzyme pyruvate decarboxylase is subject to activation by its substrate,[1-3] but the mode by which this activation occurs is unknown. The detailed mechanism for the catalytic process of the enzyme was proposed by Breslow to

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